Original Article

Homologous recombination pathway gene variants identified by tumor-only sequencing assays in lung carcinoma patients

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Background: The homologous recombination (HR) repair pathway plays a key role in double-stranded DNA break repair, and germline HR pathway gene variants are associated with increased risk of several cancers, including breast and ovarian cancer. HR deficiency is also a therapeutically targetable phenotype.

Methods: Somatic (tumour-only) sequencing was performed on 1,109 cases of lung tumors, and the pathological data were reviewed to filter for lung primary carcinomas. Cases were filtered for variants (disease-associated or of uncertain significance) in 14 HR pathway genes, including *BRCA1*, *BRCA2*, and *ATM*. The clinical, pathological and molecular data were reviewed.

Results: Sixty-one HR pathway gene variants in 56 patients with primary lung cancer were identified. Further filtering by variant allele fraction (VAF) of ≥30% identified 17 HR pathway gene variants in 17 patients. *ATM* gene variants were most the commonly identified (9/17), including two patients with c.7271T>G (p.V2424G), a variant in the germline that is associated with increased familial cancer risk. Four (4/17) patients had a family history of lung cancer, among which three patients had *ATM* gene variants suspected to be germline in origin. In three other patients with *BRCA1/2* or *PALB2* gene variants who had undergone germline testing, the variants were confirmed to be germline; lung cancer was the sentinel cancer in two of these patients with a *BRCA1* or *PALB2* variant.

Conclusions: Genomic variants in the HR repair pathway identified in tumor-only sequencing and occurring at higher VAFs (i.e., ≥30%) may suggest a germline origin. Correlating with personal and family history, a subset of these variants is also suggested to be associated with familial cancer risks. Patient age, smoking history and driver mutation status are expected to be a poor screening tool in identifying these patients. Finally, the relative enrichment for *ATM* variants in our cohort suggests a possible association between *ATM* mutation and lung cancer risk.

Keywords: Lung cancer; BRCA1; BRCA2; ATM; homologous recombination (HR)

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Introduction

The homologous recombination (HR) DNA repair pathway plays a key role in double-stranded DNA break repair. Briefly, this pathway senses double-stranded DNA breaks by the MRN (MRE11-RAD50-NBS1) complex, followed by ATM activation, extensive DNA processing (end resection, facilitated by BRCA1-PALB2-BRCA2 and RAD51 paralogs), homology search (after loading of RPA and RAD51), and subsequent homologous template-dependent repair (1-5). Somatic variants in the HR repair pathway genes, including *BRCA1* and *BRCA2* gene variants, have been identified in some non-small cell lung cancer (NSCLC) specimens, but the clinical significance of these variants remains poorly understood. By contrast, the HR pathway gene variants are of marked prognostic and predictive significance in several other cancers. Germline HR gene mutations are associated with increased cancer risk exemplified by the hereditary breast and ovarian cancer (HBOC) syndrome (6). However, many laboratories perform tumor-only sequencing, where the germline-somatic distinction can be difficult. Higher variant allele fractions in tumor (VAFs; which is the number of times a variant is detected relative to the overall number of times a genomic position is sequenced) may suggest germline origin, although confirmation through formal germline testing is always recommended (7).

Lung cancer is generally understood to be related predominantly related to environmental factors. Only a small number of germline variants have been associated with increased lung cancer risk (e.g., *EGFR* p.T790M) (8). While the American College of Medical Genetics and Genomics provides guidance on referral for cancer predisposition assessment (9), lung cancer is not addressed in the practice guideline. Lung cancer patients do not routinely undergo genetic counseling. In assessing family cancer history, significance of lung cancer cases in relatives is often unclear. In this study, we report HR pathway gene variants detected in tumors from 56 patients with NSCLC, and correlate these findings with clinical, pathological and molecular data. We present this article in accordance with the MDAR reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-749/rc).

Highlight box

Key findings

- Higher variant allele fractions (VAF) for homologous recombination (HR) repair pathway gene variants identified in lung cancers, with tumor-only sequencing, may suggest germline origin.

What is known and what is new?

- HR gene variants can be identified in lung cancer, but their clinical significance is unclear.
- A subset of HR gene variants identified in lung cancer were confirmed to be of germline origin, which may be associated with familial cancer risks.

What is the implication, and what should change now?

- Genetic counseling and followup germline testing may be of value in a subset of patients for whom HR gene variants were identified with tumor-only sequencing, depending on the VAF and/or clinical/family history.

Methods

Case selection

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was performed with the approval of University of Pennsylvania's Ethics Board (Protocol No. 834224) and the requirement for patient consent was waived by the research ethics board, considering the retrospective nature of the study. We examined all cases sequenced at the Center for Personalized Diagnostics (CPD; University of Pennsylvania, from September 2016 to October 2019) and filtered for cases of interest using the algorithm outlined in Figure S1. Cases were selected based on the tissue assignment (by referring physician) and on the sequencing panel performed on the submitted specimen (see below). In total, 1,109 cases of "lung" cancers were sequenced using our solid tumor panel, which targets 152 genes (encompassing ~0.5 Mbp of genomic DNA). The resultant mutational data were plotted as waterfall and lollipop plots using R packages (GenVisR, version 3.11). Chart reviews were performed for the 56 patients with a confirmed diagnosis of lung cancer in whom we found variants in the HR repair pathway, and abstracted for details of pathology, any germline testing data, past medical history including cigarette smoking, and family cancer history. All data analyzed are available in Table S1.

Statistical analyses

Comparisons of continuous data between two groups were performed the Mann-Whitney test. Comparison of categorical data between two groups was performed using the Fisher's exact test.

Massively parallel sequencing assays

Molecular workup for a new patient with lung cancer at the Hospital of the University of Pennsylvania has rapidly evolved over the past 10 years. The most recent version of the workup algorithm entails detection of single nucleotide variants and other small (i.e., less than approximately 30 bp) genetic alterations, using a 152-gene sequencing panel (solid panel version 2, Table S2). Fusion transcripts, including *ALK*, *RET*, *ROS1*, and *NTRK* fusions, are detected using a 55 gene-target fusion panel (Table S3), and/or fluorescence *in situ* hybridization. *MET* exon 14 skipping mutations (i.e., exons joined out of order or point mutations known to cause exon skipping events) are detected by these panels. Genetic variants detected are internally classified as disease-associated, probably disease-associated, variant of unknown significance (VUS), likely benign, or benign, and reported as either disease associated or VUS (which encompasses probably disease-associated, VUS, and likely benign). While the concepts are not formally equivalent, this classification scheme is similar to the Association for Molecular Pathology (AMP)/American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) classification system (10); disease-associated and probably disease-associated variants would be largely equivalent to AMP/American College of Medical Genetics (ACMG) tier I–II variants (i.e., therapeutic, prognostic or diagnostic significance). For example, strong clinical significance for *BRCA1/2* gene variants may be related to variants' known association with familial cancer risks, or response to PARP inhibitors, among other lines of evidence. Other variants would be classified as tier III (VUS) or IV (likely benign and benign); these latter variants were excluded from further analysis. Interpretation and tiering of variants was performed in the context of available data, including which include gene-specific (i.e., *BRCA1/2*) databases. Of note, the next generation sequencing (NGS) workflow is a tumor-only sequencing assay, and the distinction between variants of germline and somatic derivation is not made. Tumor mutational burden (TMB) was assessed using an internally developed algorithm (manuscript in preparation).

Results

Data review and case selection

The sequencing panel includes 14 genes in the HR repair pathway, namely *ATM*, *ATRX*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *MRE11A*, *NBN*, *PALB2*, *RAD50*, *RAD51*, *RAD51B*, *RAD51C*, and *RAD51D*, ranging from genes with well-established roles in HR repair (e.g., *BRCA1/2*) to genes with more recently recognized roles [e.g., *ATRX* (11)]. Following retrospective review of molecular profiling data for 1,109 cases of "lung cancers", we identified 61 disease-associated/probably disease-associated variants in 56 unique patients with NSCLC. *ATM* gene variants were most common (24/61 variants), followed by *BRCA1* (9 variants), *ATRX* (4 variants) and *BRCA2* (4 variants) (*Figure 1*). Multiple HR gene pathway variants were identified in five patients, three of whom had two disease-associated variants in the *ATM* gene. The 56 unique cases comprised 49 adenocarcinomas, one squamous cell carcinoma (SCC), and six carcinomas not otherwise specified (NOS). The patient ages ranged from 39 to 91 years (median 68) at diagnosis. Cigarette smoking history was available in 55 patients, of which 50 patients had a positive smoking history, with 5 never-smokers. More detailed smoking history was available for 41 patients, among which the average exposure was 39.5 pack-years. Among oncogenic driver variants in our 56 patients with HR repair pathway mutations, co-mutations in *KRAS* were the most common (30/56 patients), followed by *EGFR* and *MET* (2 patients each). Single cases of *ALK*, *NRAS* and *ERBB2*-driven cases were also identified. No driver genetic mutations were identified in 19 cases. TMB ranged from 0 to 34.3 mutations/megabase (average 7.4).

Variants with higher allele fractions

We filtered the variants based on VAFs—we reasoned that HR gene variants seen at higher VAFs are more likely to be of germline origin. Seventeen DNA variants from 17 patients were observed at VAFs ≥30%. While germline variants are often identified at VAF of approximately 50%, VAF of 30% was chosen to increase the sensitivity for identifying potentially germline variants, as VAFs for germline variants can range widely, related to both biologic (e.g., copy number changes) and technical (e.g., low read depth) issues. Of the 17 patients with HR pathways gene variants at ≥30% VAF, four patients had a prior personal cancer history (*Figure 2*). Family history was available for 16/17 high VAF patients, with family history information limited in one patient. By the National Comprehensive Cancer Network (NCCN) HBOC criteria (12), family history would be considered significant in two patients. That is, the patient would have met the criteria for germline testing; one such patient had a history of ovarian cancer in a 1st-degree relative, and another patient had a 2nd-degree relative with ovarian cancer. Breast cancer family history

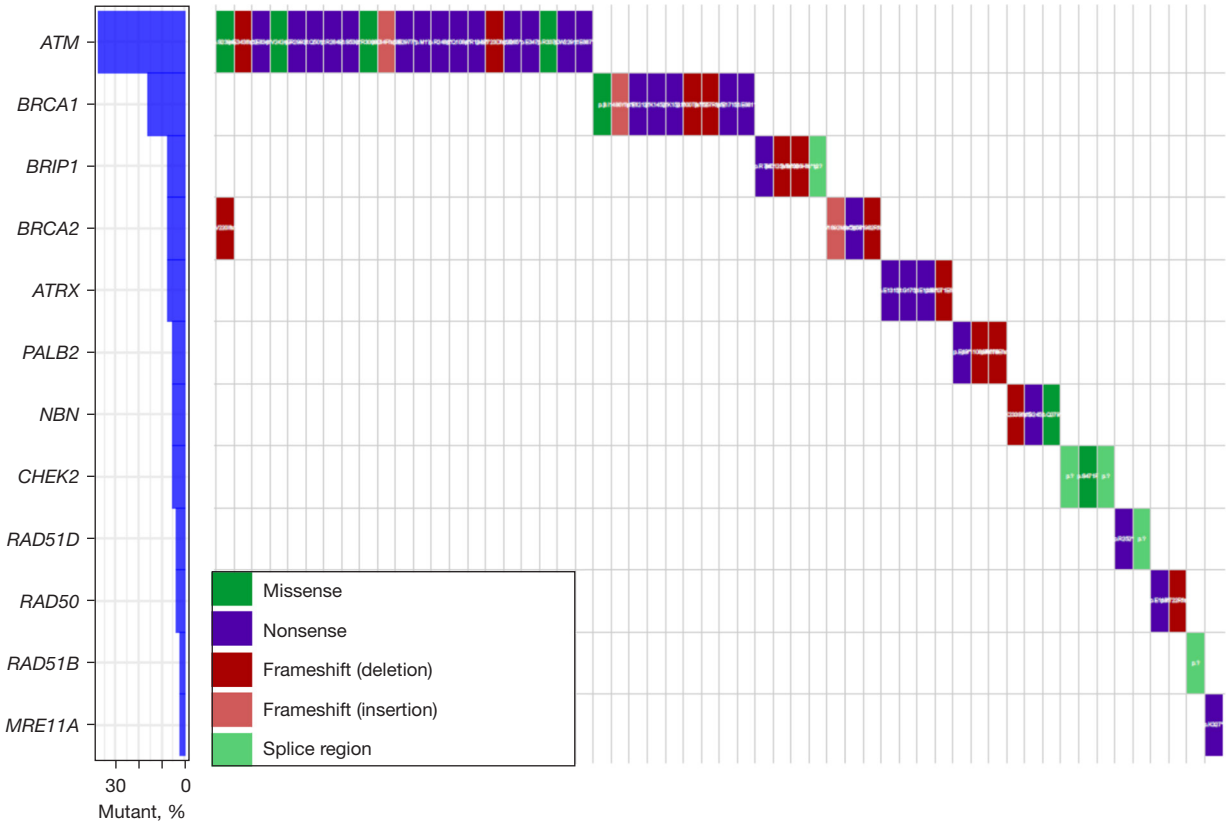

Figure 1 Waterfall plot displaying the disease-associated/probably disease-associated variants in HR pathway genes from 56 patients with NSCLC. In total, 1,109 lung cancer patients were sequenced. HR, homologous recombination; NSCLC, non-small cell lung cancer.

was positive in seven patients, 6/7 cases of which were in 1st-degree relatives, although the ages at diagnosis and cancer subtype (e.g., triple-negative) were not available. Similarly, prostate cancer family history was positive for three patients, but information regarding the specific disease attributes (Gleason grade, disease burden, clinical course) was unavailable. Family history of pancreatic cancer was positive in one patient (2nd-degree relative). In addition to the typical HBOC-associated cancers, family history of lung cancer was identified in 4/16 patients, all such patients having at least one affected 1st-degree family member.

Of the 14 HR pathway genes examined, only variants in seven genes were observed at a VAF ≥30%, with *ATM* gene variants being most common (9/17 patients). In the germline setting, *ATM* is generally considered an intermediate cancer risk gene, with the exception of *ATM* p.V2424G (NM_000051.4: c.7271T>G) that is associated with higher cancer risk when compared to other *ATM* variants (13). *ATM* p.V2424G was observed in two patients at VAFs of 55% and 77%; both patients had a history of breast cancer in their families. Seven other *ATM* disease-associated/probably disease-associated variants were identified, comprised largely of disruptive frameshift and nonsense variants. One *CHEK2* variant (NM_001005735.1: c.1412C>T; p.S471F, equivalent to NM_007194.4: c.1283C>T) stood out from the other variants, occurring in a patient from a family with nine members with a cancer history.

High VAF HR gene variants and correlations with germline/repeat somatic sequencing results

Three patients underwent germline testing, which was initiated based on the somatic sequencing results. All three patients were positive for pathogenic germline variants (*Table 1*). Among the 14 HR genes included in our study, the highest cancer risks are associated with *BRCA1*, *BRCA2*, and *PALB2* (14-16), and a germline pathogenic variant was identified in each of the high-risk genes. Examining the somatic sequencing results, *BRCA1* and *BRCA2* variants were observed at VAFs of 69% and 65%, respectively, suggesting

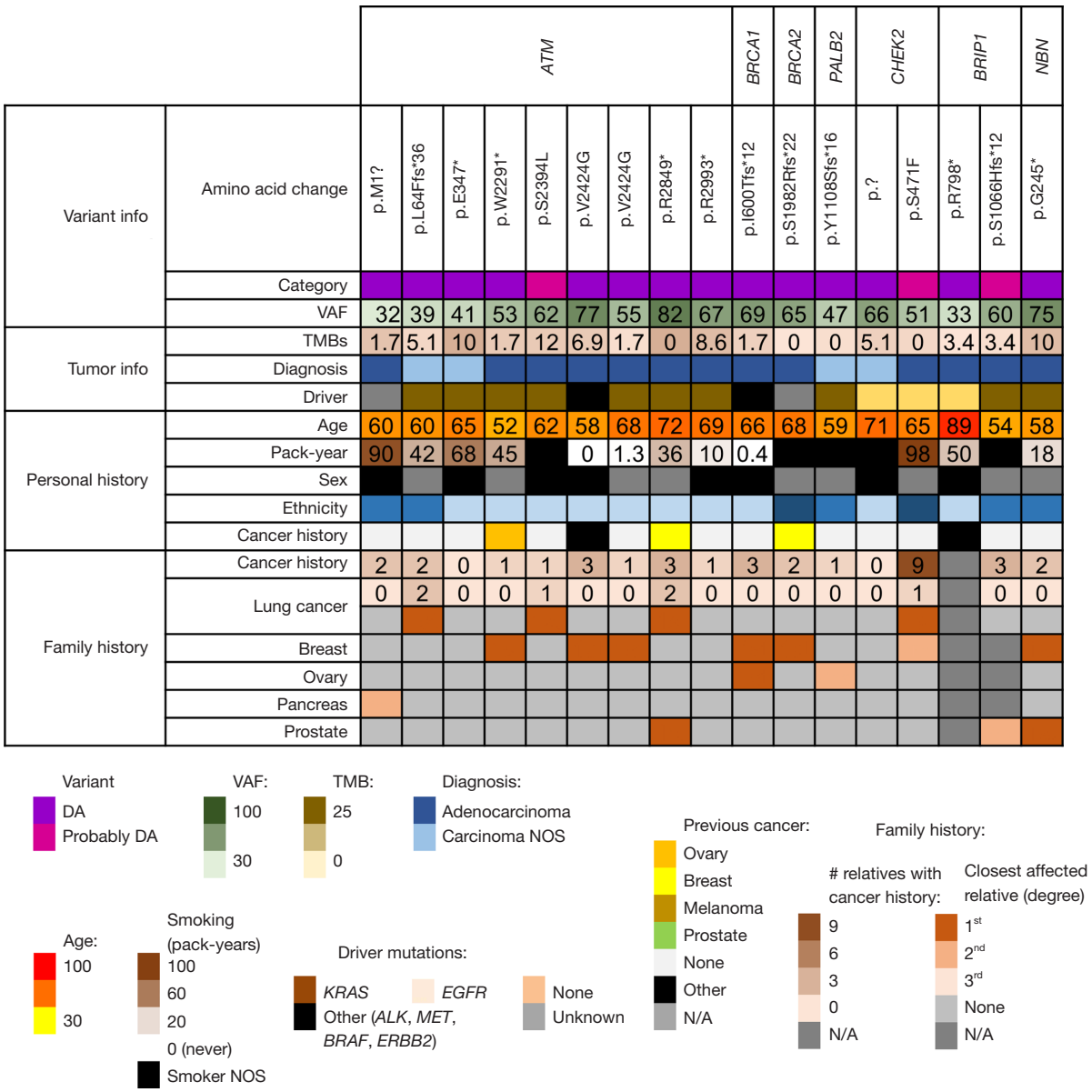

Figure 2 Seventeen HR pathway gene variants observed at VAF ≥30%, with tandem patient and family history information. DA, disease-associated; VAF, variant allele fraction; TMB, tumor mutational burden; NOS, not otherwise specified; N/A, not available; HR, homologous recombination.

that loss-of-heterozygosity (LOH) may have occurred in the tumors. In two patients, lung cancer was their first (sentinel) cancer, with no personal history of other cancers. One *BRCA1* disease-associated variant (NM_007300.4: c.1799delT; p.I600Tfs*12) was confirmed to be germline in a patient who presented at age 66 with lung cancer; the family cancer history was notable for ovarian and breast cancer in 1st degree relatives (mother and sister, respectively). In a patient with a germline *BRCA2* variant (NM_000059.4: c.5946delT; p.S1982Rfs*22), the past medical history was significant for a previous triple-negative breast carcinoma that preceded the lung cancer by 12 years. A *PALB2* variant (NM_024675.4: c.3323delA; p.Y1108Sfs*16) was confirmed to be germline in a patient who presented with lung cancer at age 59; family cancer history was notable for ovarian cancer in her maternal grandmother. All three patients had a cigarette smoking

Table 1 Germline testing results and corresponding family history[#]

Age/sex (smoking history)	Germline genetics (annotation)	Clinical history
66M (0.4 PY)	*BRCA1* NM_007300.4: c.1799delT (p.I600fs*12, pathogenic)	Personal: none
		Family: ovarian cancer (mother@47), breast (sister@62), mesothelioma (father@84)
68F ("Former", PY NOS)	*BRCA2* NM_000059.4: c.5946delT (p.S1982Rfs*22, pathogenic)	Personal: breast carcinoma (56 years, triple negative), ovarian fibroma (47 years)
		Family: breast (mother), uterus (mother)
59F ("Former", PY NOS)	*PALB2* NM_024675.4: c.3323delA (p.Tyr1108Serfs*16, pathogenic)	Personal: none
		Family: ovarian cancer (maternal grandmother)

[#], germline variant terminology differs from somatic variant classification terminology. However, in this context, "pathogenic" germline variant can be considered being equivalent to "disease-associated". @: diagnosed at age. M, male; PY, pack-year; F, female; NOS, not otherwise specified.

Table 2 HR gene variants detected at ≥30% VAF from three patients with repeat sequencing results[#]

Variant	Detected on repeat assay?	VAF		Time between samples	Shared driver gene?
		Initial	Repeat		
ATM NM_000051.4: c.7271T>G (p.V2424G)	Yes	77%	78%	3 years	N/A*
ATM NM_000051.4: c.7271T>G (p.V2424G)	Yes	55%	56%	1 year	No (*KRAS*)**
BRIP1 NM_032043.2: c.3196delT (p.S1066Hfs*12)	Yes	60%	80%	1 year	Yes (*KRAS*)

[#], the table compares data from metachronous, potentially independent (*vs.* recurrent) tumors that underwent molecular profiling from three different patients; *, N/A: not applicable—no driver genetic event identified in either samples; **, two different *KRAS* driver mutations (c.35G>A; p.G12D and c.34G>T; p.G12C), consistent with two independent tumors. HR, homologous recombination; VAF, variant allele fraction.

history, with the pack-years history unknown in two patients.

Three other patients had undergone repeat somatic (tumor-only) sequencing without germline testing (*Table 2*). If HR pathway gene variants are detected in two metachronous tumors from the same patient, this increases the likelihood that the variants are of germline origin. For all three patients who had undergone repeat somatic sequencing, the HR pathway gene variants were consistently detected. Two of these patients had the *ATM* p.V2424G variant, and one patient had a *BRIP* gene variant. In one patient, the *ATM* p.V2424G variant was identified in two definitively metachronous, independent tumors that harbored two different *KRAS* mutations, lending further support to the *ATM* gene variant being germline in the patient.

Comparison of patients with high VAF vs. low VAF tumors

The above data suggested at least six of our patients (who had undergone germline or repeat somatic sequencing) likely harbor germline variants in HR pathway genes, confirming the potential utility of a VAF ≥30% as a screen to identify potential germline variants. By contrast, the HR pathway genes observed at low VAFs are more likely to be truncal, possibly passenger mutations, acquired during tumor progression/evolution. Given that this distinction may be helpful in patient selection for germline testing, we compared the patients with high *vs.* low VAF variants for clinicopathological features that may further aid in the distinction. When we compared the patients with HR gene variants identified at low or high VAFs, the two groups did not differ with respect to age at diagnosis, proportion of 20+ pack-year cigarette smokers, or mean pack-years (where the information was available, *Table 3*). Also, their tumors did not differ significantly with respect to proportion with/without targetable genetic mutations; *KRAS* was the most common oncogenic driver mutation in both groups.

Table 3 Comparison between patients with/without high VAF (≥30%) HR gene variants

Groups features	VAF <30%	VAF ≥30%	P values (test)
Number	39 patients	17 patients	
Age at diagnosis	Mean age 67.8 years (SD 10.7)	Mean 64.5 years (SD 8.9)	0.1031 (Mann-Whitney)
Cigarette smoking history	34/38 (89.5%) positive history	16/17 (94.1%) positive	1.000 (Fisher's exact test)
	38.7 mean pack-years (SD 21.1, n=30)**	41.7 mean pack-years (SD 28.4, n=11)**	0.9283 (Mann-Whitney)
	26/30 (86.7%) with ≥20 pack year smoking history	7/11 (63.6%) with ≥20 pack year smoking history	0.1777 (Fisher's exact test)
Driver			
Non-targetable*	20 *KRAS*, 1 *NRAS*, 7 none, 7 unknown	10 *KRAS*, 3 none, 2 unknown	1.000 (Fisher's exact test)
Targetable	2 *EGFR*, 2 *MET*	1 *ALK*, 1 *ERBB2*	
TMBs (mutations per megabase, /Mbp), mean ± SD	8.8±6.2	4.2±6.6	0.0036 (Mann Whitney)

Note: *KRAS* variants include the *KRAS* p.G12C variant, which was not a targetable gene variant at the time of patient work-up. *, generally associated with little or no smoking history; **, n indicates the number of patients with known pack-years. VAF, variant allele fraction; HR, homologous recombination; SD, standard deviation; TMB, tumor mutational burden.

Interestingly, the TMBs were significantly lower in the VAF ≥30% group (Mann-Whitney P=0.0036), although the ranges were wide for both groups (0 to 12.0/Mbp for VAF ≥30%, and 0 to 34.3/Mbp for VAF <30%). Therefore, none of the conventional clinical, pathological or molecular attributes appeared to be a useful flag for potential germline variants in HR pathway genes, with the exception of differences in VAFs and TMBs.

Discussion

We report HR pathway gene variants (categorized as disease-associated or probably disease-associated at our institution) in 56/1,109 (5.0%) lung cancer specimens, with the gene variants being observed over a wide range of VAFs. Most variants (44/61, 72.1%) were observed at VAF <30%; such variants are likely passenger mutations acquired with tumor evolution, unlikely to be of germline origin, and unlikely to be associated with LOH. The remaining 17 patients' tumors harbored HR gene variants at VAFs ≥30%, where the higher VAFs suggested that some of the variants may be germline in origin. Indeed, a subset of these variants, which included disease-associated *BRCA1/2*, or *PALB2* mutations, as well as *ATM* variants, were associated with even higher VAFs and often with significant personal and/or family cancer history. While the VAF cut-off of 30% was useful in our study, its sensitivity and specificity in use for flagging potentially germline variants are to be studied further.

Genetic counseling for lung cancer patients can be challenging for numerous reasons, including the generally short survival span for these patients; the five-year survival for lung cancer (~21%) differs drastically from breast cancer (90%) [Surveillance, Epidemiology, and End Results (SEER) data, accessed June 2020]. Discussion of "familial lung cancer" is also limited in the literature, with the *EGFR* p.T790M variant as the sole well-established risk-elevating allele (8). Many lung cancer patients also have a cigarette smoking history, which also may be a shared risk factor amongst family members through first and/or second-hand smoking. Our data demonstrate that lung cancer patients can harbor pathogenic, germline HR pathway gene variants and that somatic testing can point to their existence. Our data suggest that patient characteristics such as older age and cigarette smoking history are unlikely to be helpful in identifying germline HR pathway variants. As such, germline testing should be considered in all patients in whom pathogenic high-VAF variants are identified by somatic testing. Although only three patients with HR pathway gene variants at high VAF underwent germline testing, it is noteworthy that all three were positive for HR gene variants, suggesting that lung cancer can be the sentinel cancer in a patient with a pathogenic *BRCA1* or

PALB2 gene variant.

Among the 14 HR genes analyzed in our study, *ATM* variants were the most common, which may be related to the large size of the *ATM* gene (146,619 bp, based on hg19). However, a few of the other HR pathway genes are comparable in size, including *BRCA1* (125,951 bp). Many of the observed *ATM* gene variants in our patients with lung cancer are highly suspicious for germline origin, based on the high VAFs and clinical histories. The repeat somatic sequencing data are particularly suggestive, especially in the patient with two metachronous, independent tumors harboring the same *ATM* gene variant. Several *ATM* single-nucleotide polymorphisms have been linked to increased lung cancer risk, including rs189037 (c.-111G>A on the transcript NM_000051.3), rs664677 (c.3078-77C>T) and rs664143 (c.8850+60A>G) (17,18). More recently, a case-control association study examining 1,083 lung adenocarcinoma patients identified rare, deleterious, germline *ATM* variants more frequently in patients with lung adenocarcinoma (odds ratio of 4.6) (19). The *ATM* gene variants identified in that study did not overlap with the variants identified in our study, partly related to our exon-centric sequencing panel design. Our data also support the concept that *ATM* gene variants may be related to increased familial lung cancer risk, likely further exacerbated by cigarette smoking.

Conclusions

In summary, we demonstrate that pathogenic HR pathway gene variants are identified in a subset of patients with primary lung cancer undergoing routine tumor-only sequencing as part of standard of care. Among the patients in whom these HR pathway variants are observed at higher VAFs, including patients with *ATM* gene variants, further germline testing may be of value to the patients and their relatives, regardless of patient's age at diagnosis, and cigarette smoking history.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-749/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-749/dss

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-749/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-749/coif). JJR reports holding stock/stock options in Pfizer and Eli Lilly personally and serves as a member representative on an external advisory stakeholder for a grant entitled "Randomized trial of universal *vs.* guideline-directed germline testing among young adults with cancer" which is an NCI Cancer Moonshot Approaches to Identify and Care for Individuals with Inherited Cancer Syndromes (U01). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was performed with the approval of University of Pennsylvania's Ethics Board (Protocol No. 834224) and the requirement for patient consent was waived by the research ethics board, considering the retrospective nature of the study.

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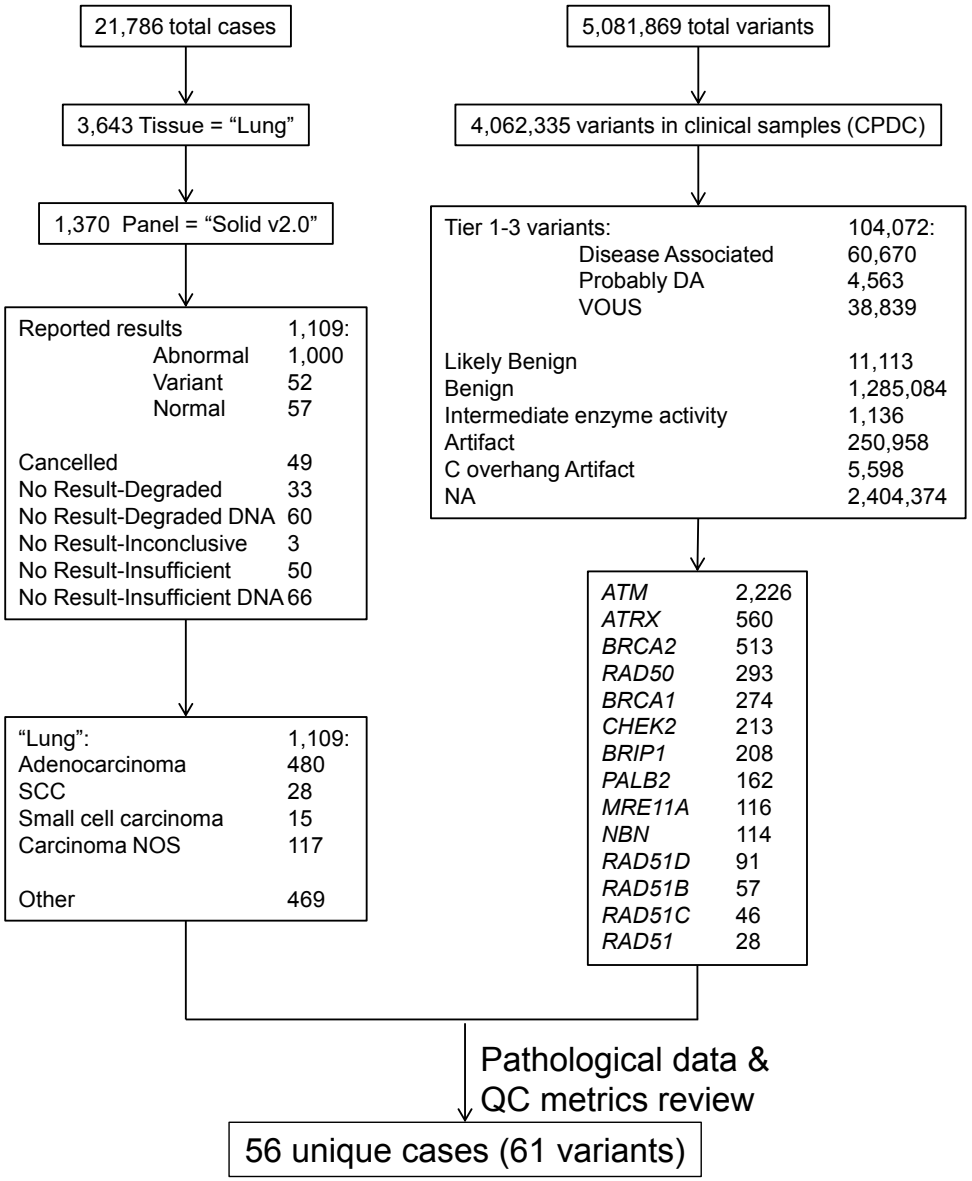

Figure S1 Algorithm used for filtering of cases of interest. All cases were sequenced at the Center for Personalized Diagnostics (CPD; University of Pennsylvania), from September 2016 to October 2019. DA, disease associated; VOUS (VUS), variant of uncertain significance; NA, not available/applicable; SCC, squamous cell carcinoma; NOS, not otherwise specified; QC, quality control.

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Table S1 Lung cancer family history and somatic testing results

Age/sex (smoking)	Somatic alteration (amino acid change, VAF, classification)	Family cancer history[#]
60F (42 PY)	*ATM* NM_000051.4: c.192delAinsTG (p.L64Ffs*36, 39%, DA)	Lung (father@55, sister@59)
72F (36 PY)	*ATM* NM_000051.4: c.8545C>T (p.R2849*, 82%, DA)	Prostate (father), lung (brother, nephew)
62M (PY unknown)	*ATM* NM_000051.4: c.7181C>T (p.S2394L, 62%, probably DA)	Lung (sister)
65F (98 PY)	*CHEK2* NM_001005735.2: c.1412C>T (p.S471F, 51%, probably DA)	Kidney (brother), lung (father), colon (maternal GF, maternal GM, 2 maternal uncles), breast (maternal GM), lymphoma (mother), leukemia (sister)
45M (never)	*ATM* NM_000051.4: c.2179G>C (p.G727R, 47%, VUS)	Breast (mother@56, paternal GM), pancreas (mother@56), lung (paternal GM)
67F (17 PY)	*ATM* NM_000051.4: c.2930G>T (p.C977F, 45%, VUS)	Lung (father), breast (mother), cervix (mother), thyroid (mother)
52F (15 PY)	*ATM* NM_000051.4: c.5489T>C (p.M1830T, 59%, VUS)	Lung (NOS)
65M (27 PY)	*ATM* NM_000051.4: c.6998C>T (p.T2333I, 70%, VUS)	Lung (son-small cell), colon (father, maternal GF, paternal GF), prostate (father, paternal GF)
55F (35 PY)	*ATM* NM_000051.4: c.9031A>G (p.M3011V, 52%, VUS)	Lung (2 brothers, father)
54F (40 PY)	*ATRX* (NM_000489.6): c.2933C>T (p.S978F, 52%, VUS)	Lung (father), skin (sister), breast (paternal GM), colorectal (maternal GM)
56F (34 PY)	*BRCA2* NM_000059.4: c.2716A>G (p.T906A, 60%, VUS)	Lung(mother), breast (maternal GM)
41F (38 PY)	*BRCA2* NM_000059.4: c.4436G>C (p.S1479T, 40%, VUS)	Lung (2 maternal aunts), GYN (maternal aunt)
54F (never)	*BRCA2* NM_000059.4: c.8732C>G (p.A2911G, 33%, VUS)	Lung (father), colon (maternal GF), lymphoma (maternal GM)
62F (never)	*BRCA2* NM_000059.4: c.9391T>C (p.S3131P, 75%, VUS)	Breast (paternal cousin@53, paternal aunt@85), lung (maternal aunt@82), leukemia (mother@39), head and neck cancer (brother@62)
55M (PY unknown)	*CHEK2* NM_001005735.2: c.1556C>T (p.T519M, 44%, VUS)	Kidney (father), lung (father)
72F (50 PY)	*MRE11A* NM_005591.4: c.1972A>G (p.T658A, 48%, VUS)	Lung(father), ovary(mother)

[#], ages at diagnosis are indicated, where known. @: diagnosed at age. VAF, variant allele fraction; PY, pack-year cigarette smoking; F, female; M, male; DA, disease-associated genetic variant; GF, grandfather; GM, grandmother; VUS, variant of unknown significance; NOS, not otherwise specified; GYN, unspecified gynecological cancer.

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Table S2 152 genes sequenced at the Center for Personalized Diagnostics

ABL1
APC
ATM
BRCA1
CCND1
CDK4
CRKL
DNMT3A
ERBB3
ESR2
FGFR2
GATA3
H3F3A
JAK2
KDR
MAP2K2
MCL1
MET
MSH6
NF1
NKX2-1
EP300
PIK3CA
PTPN11
RAD51B
RET
SLIT2
SRC
SYK
TSC1
WT1
AKT1
AR
ATRX
BRCA2

Table S2 (*continued*)

CCND2
CDK6
CSF1R
EGFR
ERBB4
EZH2
FGFR3
GNA11
IDH1
JAK3
KIT
MAP2K4
MDM2
MITF
MTOR
NF2
NOTCH1
PAK1
PIK3CB
RAB35
RAD51C
RHOA
SMAD4
STAG2
TET2
TSC2
XRCC2
AKT2
ARAF
AURKA
BRIP
CCND3
CDKN2A
CTNNB1
EIF1Ax

Table S2 (*continued*)

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Table S2 (*continued*)

ERCC2
FBXW7
FGFR4
GNAQ
IDH2
KDM5A
KMT2C
MAPK1
MDM4
MLH1
MYC
NTRK1
NOTCH2
PALB2
PIK3R1
RAC1
RAD51D
RNF43
SMARCA4
STK11
TGFBR2
TSHR
AKT3
ARID1A
BAP1
BTK
CCNE1
CHEK2
DAXX
EPHA3
ERG
FGF3
FLT3
GNAS
IGF1R

Table S2 (*continued*)

Table S2 (*continued*)

KDM5C
KRAS
MAPK3
MED12
MRE11A
MYCN
NTRK2
NOTCH3
PBRM1
PTCH1
RAD50
RAF1
SETD2
SMO
SUFU
TP53
U2AF1
ALK
ARID2
BRAF
CBP
CDH1
CIC
DDR2
ERBB2
ESR1
FGFR1
FUBP1
HRAS
JAK1
KDM6A
MAP2K1
MAX
MEN1
MSH2

Table S2 (*continued*)

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Table S2 (*continued*)

NBN
NTRK3
NRAS
PDGFRA
PTEN
RAD51
RB1
SF3B1
SPOP
SUZ12
TRAF7
VHL

Table S3 55 gene targets for fusion transcript panel assay

AKT1
CALCA
EGFR3
EWSR1
FUS
KRT7
NRG1
PIK3CA
RAF1
STAT6
TFG
ALK
CAMTA1
EPC1
FGFR1
GLI1
MEAF6
NTRK1
PLAG1
RET

Table S3 (*continued*)

TAF15
THADA
AXL
CCNB3
ERBB2
FGFR2
HMGA2
MET4
NTRK2
PMS2
ROS1
TCF12
TMPRSS2
BCOR
CCND1
ERG
FGFR3
JAZF1
MKL2
NTRK3
PPARG
SLC5A5
TERT
USP6
BRAF
CIC
ESR1
FOXO1
KRT20
NCOA2
PDGFB
PTH
SS18
TFE3
YWHAE

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